Zeiss LSM 510 Confocal Microscope Training Notebook

The Center for Nanotechnology
NanoTech User Facility

September 2007

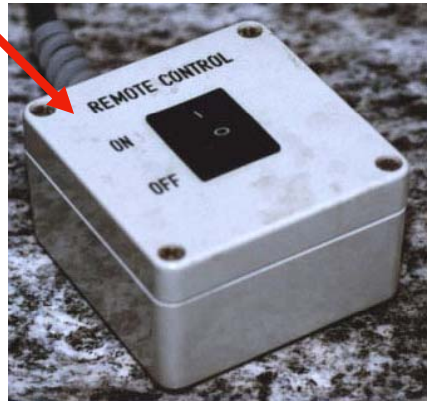
A. Turn on the System

1) Turn ON the FluoroArc mercury lamp. (Power supply is located under the vibration table)

   – The simplest rule for the Hg lamp is to leave it OFF unless you need it, but to leave it ON at the end of your session if it is still ON, so that the next users do not have to wait if they need it.

2) Turn ON the system with the remote control switch on the monitor desk (powers microscope and the visible lasers). Allow enough time for system to initialize before starting software (about 30 seconds).

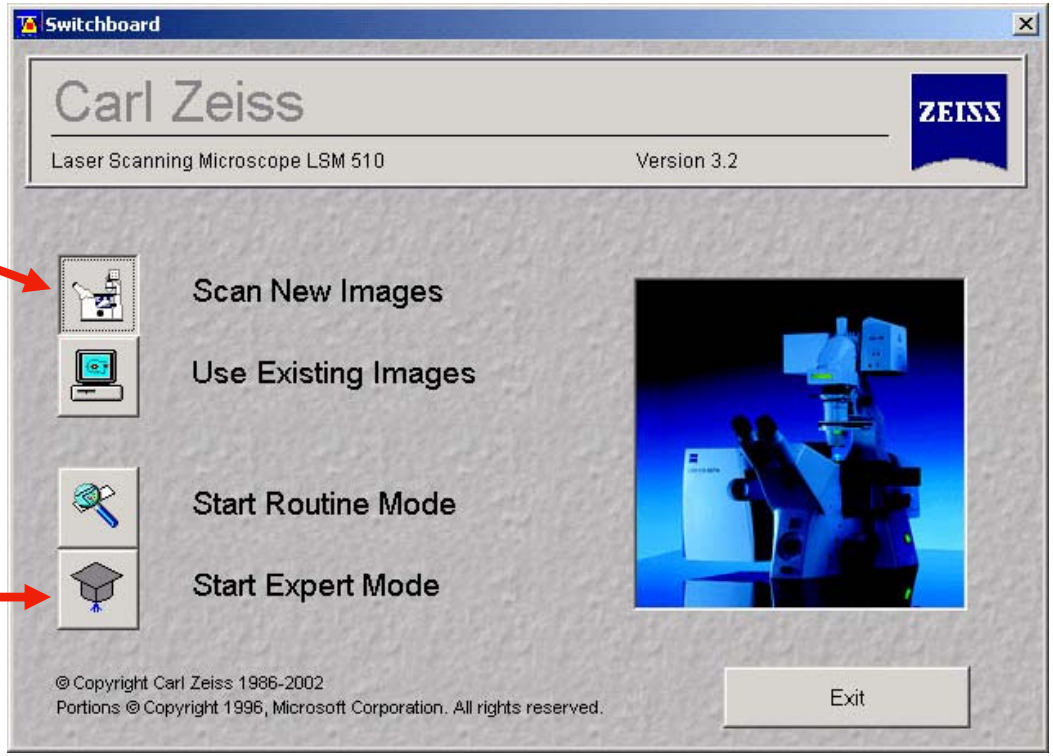
3) Start LSM 510 software (click on icon).



B. Start Operating Software

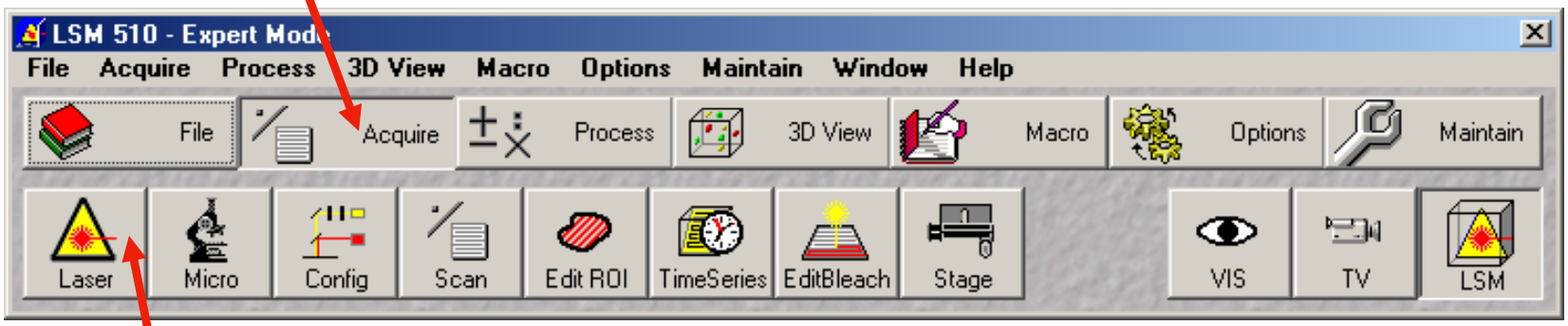
1) Select SCAN NEW IMAGES (otherwise software will work as a viewer only).

2) Click START EXPERT MODE .



C. Turn on the Lasers

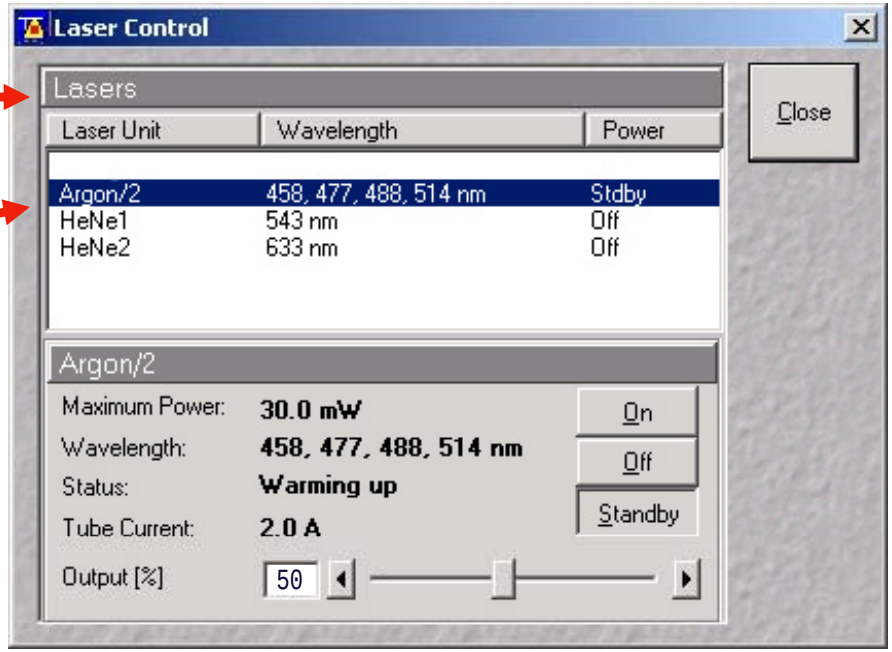
1) Select ACQUIRE.



2) Select LASER (opens laser control panel).

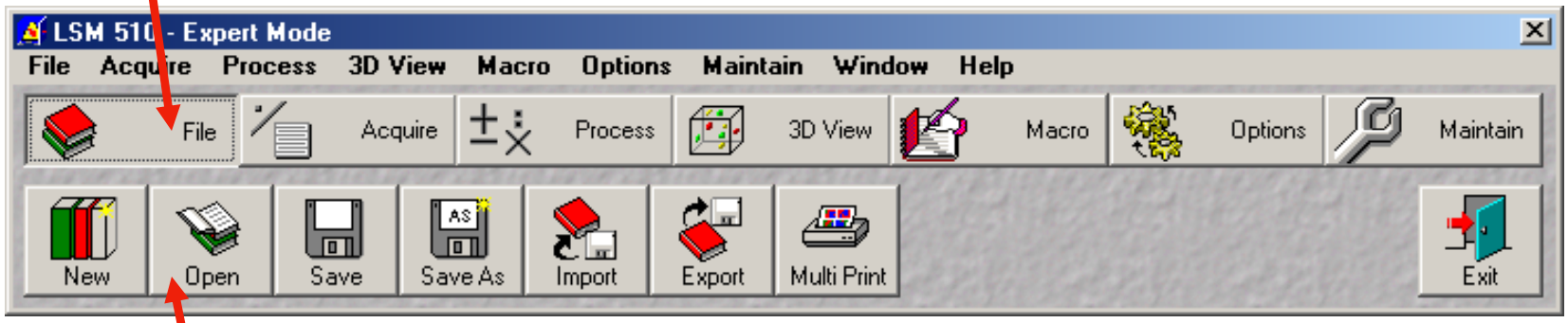
3) Click STANDBY for the Argon laser (458, 477, 488, 514 nm) and allow it to warm up. When "Ready", click ON & set output to 50% (between 5.5 and 6.5 A).

4) If needed, highlight HeNe 543 and 633 nm and click ON.



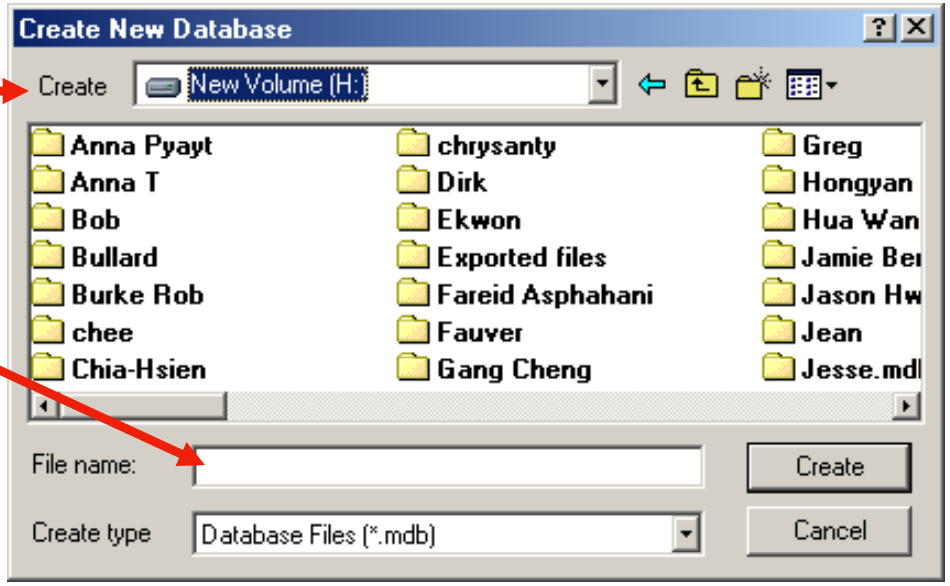
D. Set up a User Database

1) Select FILE.



2) Click NEW. In the Create New Database window, select drive G:\. Create a new directory if needed.

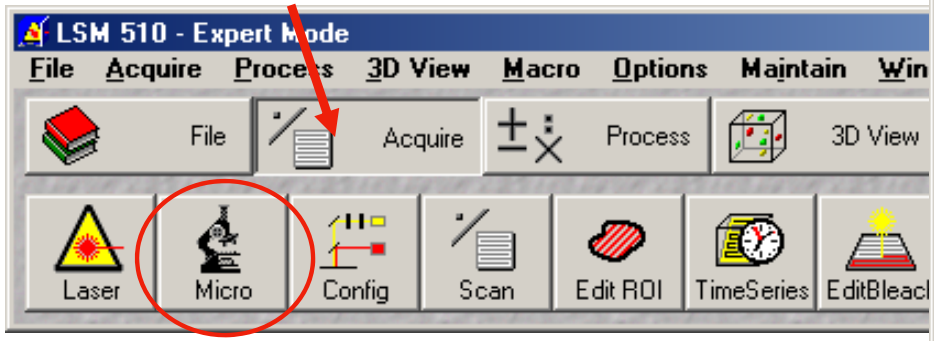
3) Under "File Name" enter a name for your database. Click "Create". A new (empty) database will be displayed.



NOTE: Temporary user's databases should be created on Drive G:. Create a new database for each session and then transfer the data to CD or DVD when you are finished . This will prevent the hard drive from filling up too quickly.

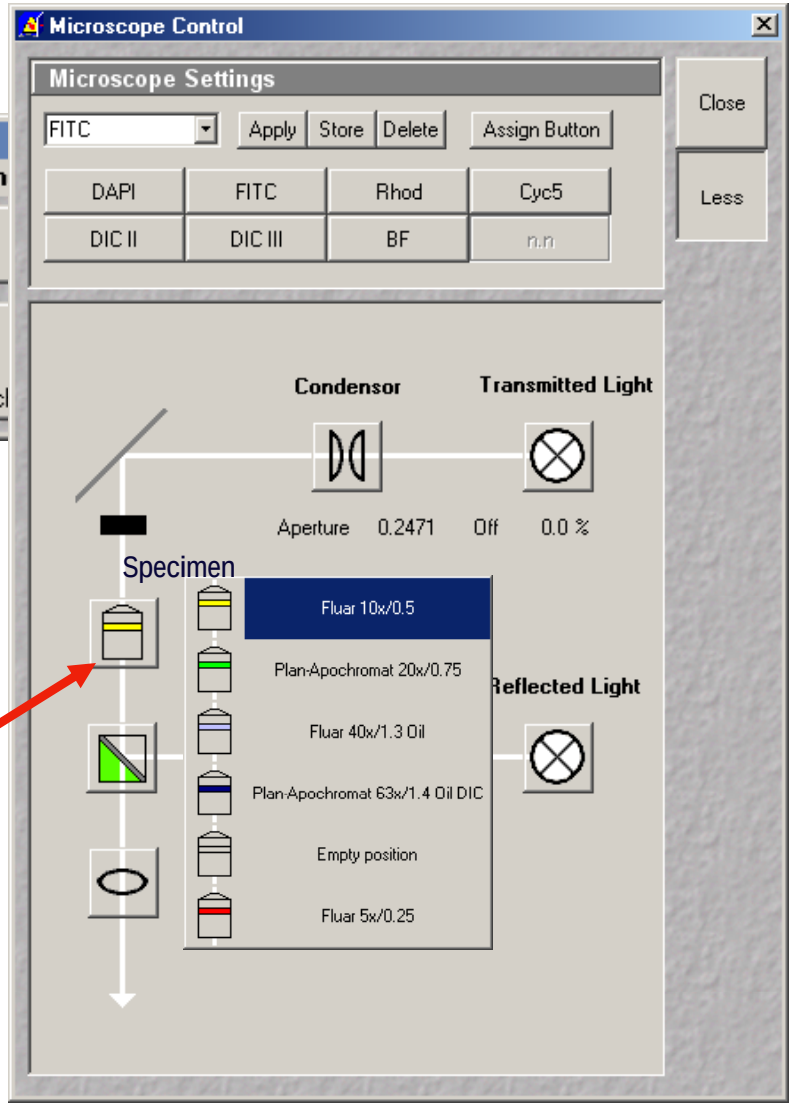
E. View Specimen at the Microscope (VIS mode)

1) Select ACQUIRE, and then click MICRO on main toolbar.



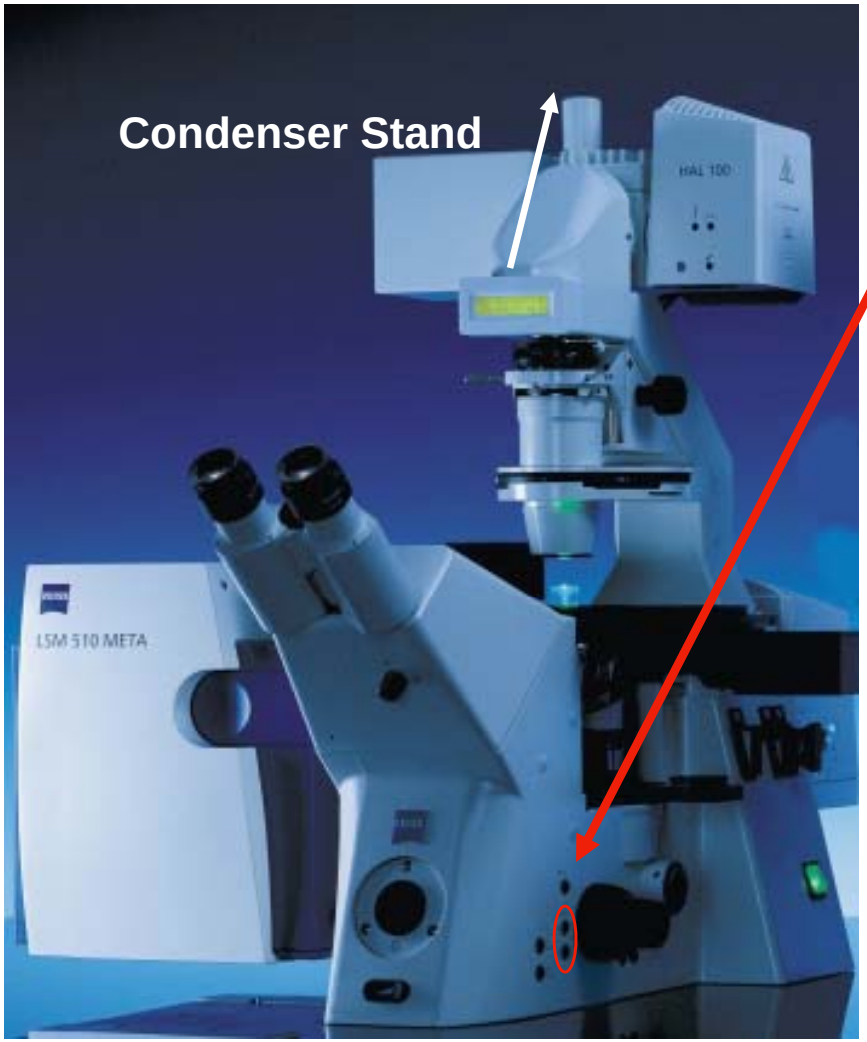
2) Toggle between VIS (visible light) and LSM (scanning mode) on the main tool bar.

3) Select OBJECTIVE on the microscope control panel.



Note: There are currently 5 objectives - three dry (5x, 10x and 20x) and two oil immersion (40x and 63x).

E-1. Load Sample at the Microscope



1) Make sure objective is lowered (use focus buttons) before putting a slide on the stage.

2) If using an immersion objective, apply a *small* amount of the immersion fluid to the objective and place sample on stage with coverslip facing down.

3) Find specimen. Use joystick for fast X/Y translation of the stage. For slow translation, press "X" or "Y" button, and rotate wheel.

4) Adjust light intensity with front switch if necessary.

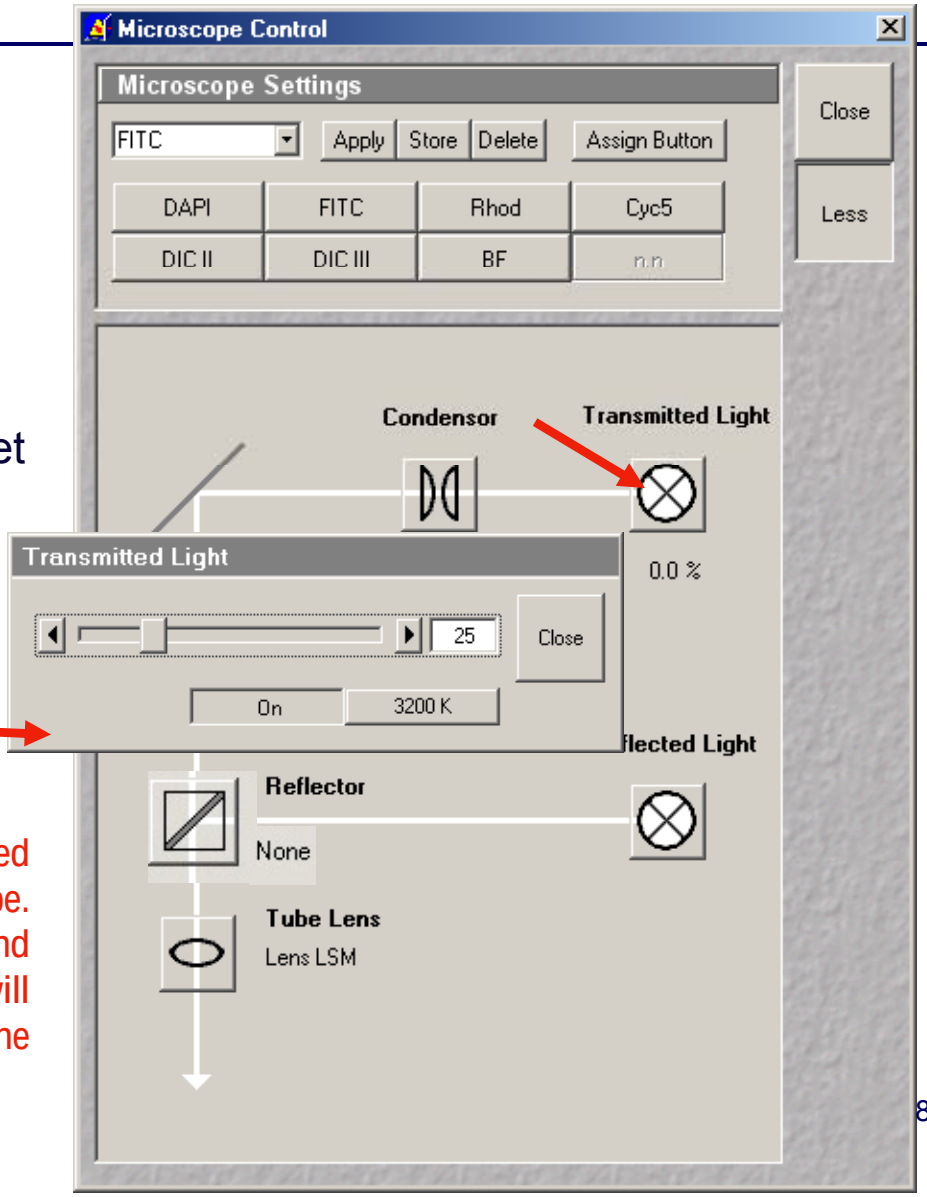
NOTE: You can gently push back the condenser stand before placing samples on the stage. Hold the condenser tower by its metal rod handle on top, not by grabbing the condenser, lamp housing, or PMT. Lower gently as well (slamming it down can damage the microscope!).

E-2. View Specimen using Transmitted Light (Bright Field)

1) Click the VIS button. This will automatically set the microscope for transmitted light viewing. Make sure Microscope Control panel is open.

2) Select Bright Field (BF) from the drop down menu (Reflector turret should be set to NONE.)

3) Click onto TRANSMITTED LIGHT and move the slider to set the intensity of the HAL illumination.



NOTE: Most microscope operations can be controlled both with the LSM 510 software, AND at the microscope. The buttons on the Axiovert 200M are well labeled, and generally self-explanatory. Pressing most buttons will display the specified action or setting on the microscope's LCD screen.

E-3. View Specimen using Fluorescence or Reflective BF

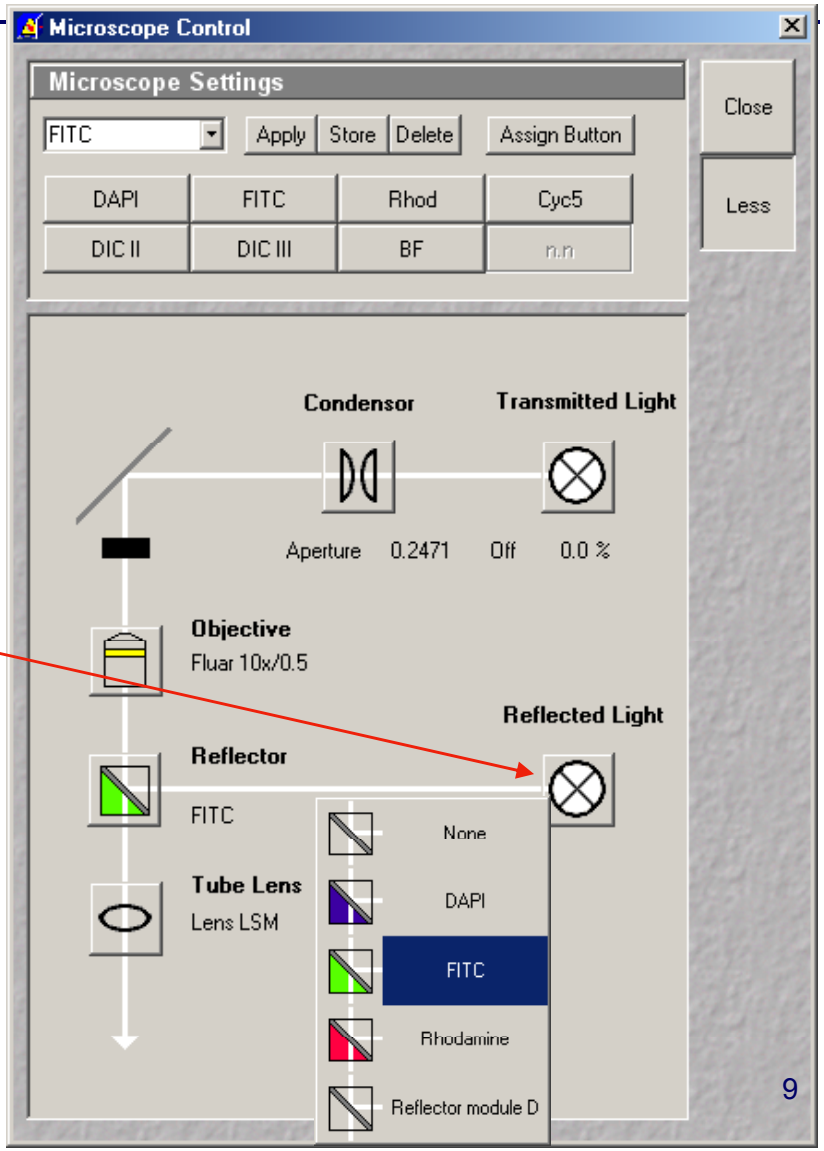
Make sure the VIS button is selected and the Microscope Control panel is open.

1) Fluorescence

- Make sure Hg lamp is selected and ON by selecting the REFELCTED LIGHT button.
- Select the appropriate filter set (REFLECTOR) by clicking the correct icon.
- Click onto the REFLECTED LIGHT button to open the shutter.

2) Reflective Bright Field (alternates with CY5)

- Turn on Reflective BF HAL lamp power supply behind microscope and manually switch from the Hg lamp to the reflective HAL lamp
- Set REFLECTOR to “BF/DIC”.
- Click onto the REFLECTED LIGHT button to open the shutter.



F. Acquire a Confocal Image

The confocal can be used in **SINGLE TRACK** (one or several channels acquired SIMULTANEOUSLY), or **MULTI TRACK**, which consists of one or several sets of single tracks, acquired SEQUENTIALLY.

SINGLE TRACK
Use for single, double and triple labeling

↓

ADVANTAGES
Faster image acquisition (simultaneous scanning only)

↓

DISADVANTAGES
Cross talk between channels.

MULTI TRACK:
Use for double or triple labeling

↓

ADVANTAGES
For an active track, only one detector/laser is switched on. Significantly reduces cross talk.

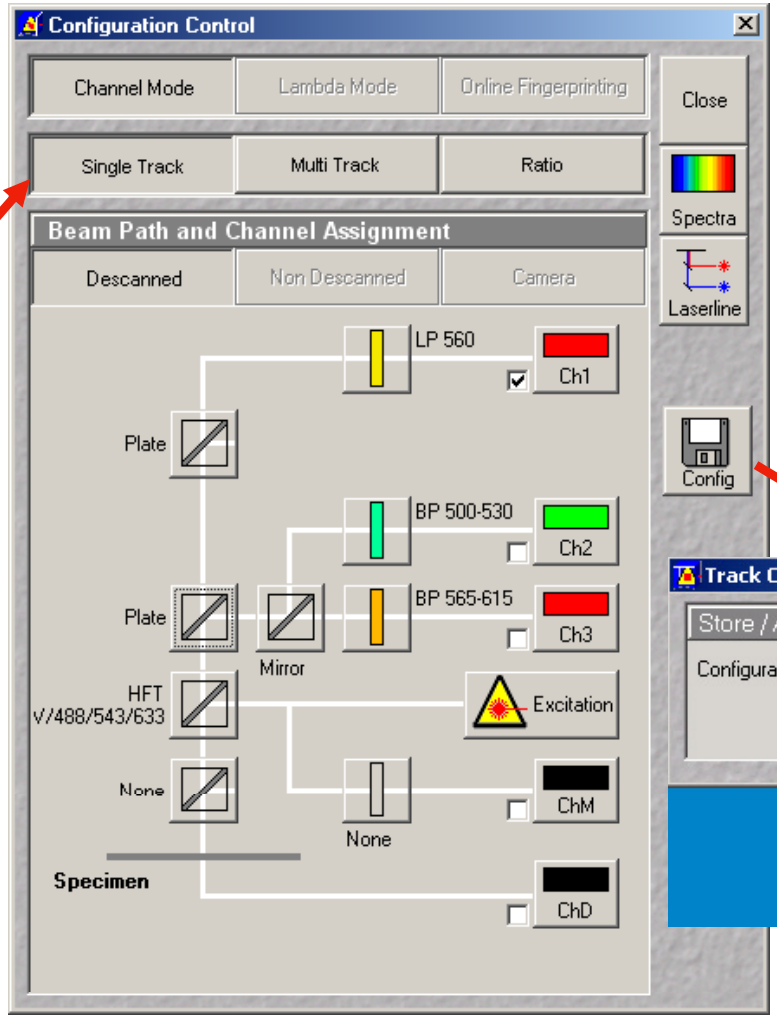
↓

DISADVANTAGES:
Slower image acquisition (Sequential scanning, line by line or frame by frame).

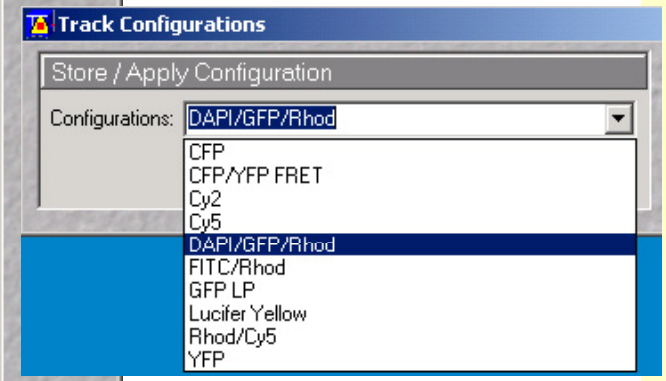
F-1. Acquire an Image in Single Track Mode

1) Select the CONFIG button on the main toolbar.

2) Select SINGLE TRACK.



3) Select CONFIG. This will display a track list menu. Select desired configuration and APPLY. You can also design your own or ask the NTUF staff for assistance.



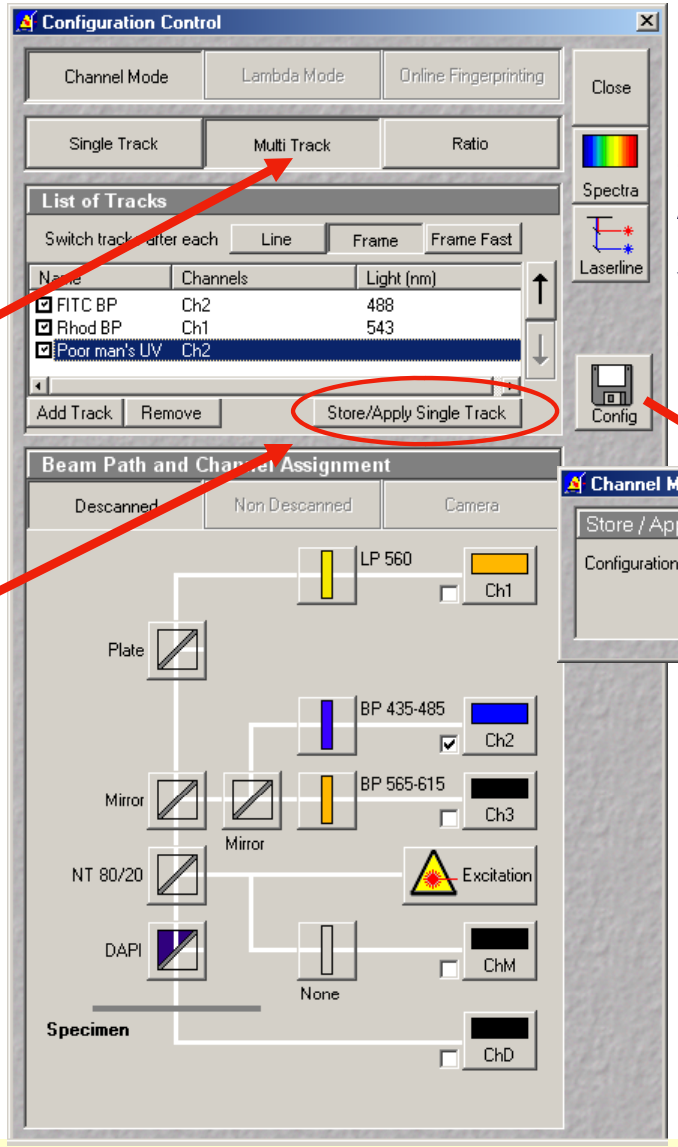
NOTE: Once you have a configuration that works for you, save it with a personal name for future use. PLEASE don't overwrite the existing configurations. You can also use your previously stored images to recall specific configurations and settings for re-use.

F-2. Acquire an Image in Multi Track Mode

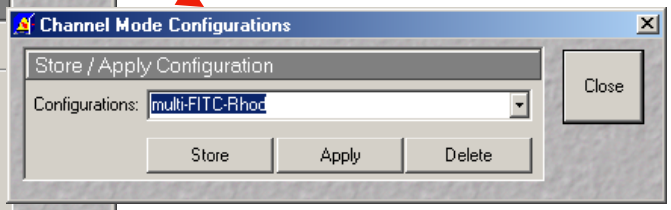
1) Select the CONFIG button on the main toolbar.

2) Select MULTI TRACK.

4) This button stores only the highlighted single track or applies a single track.



3) Select CONFIG. This will display a track list menu. Again, select desired configuration and APPLY. You can design your own or ask the NTUF staff for assistance.



G-1. Collect an Image

1) FIND opens a new image window and automatically pre-adjusts detector sensitivity.

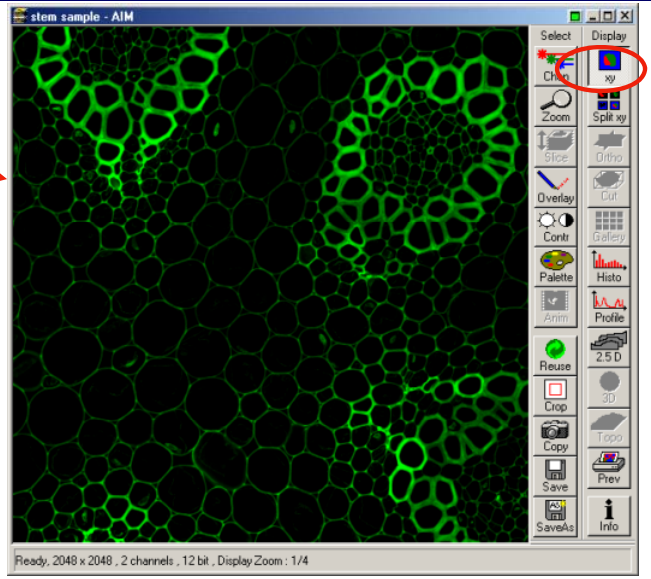
2) Use FAST XY for continuous fast scanning. Once an image is collected, be sure to adjust the *focus.* Adjust focus and X-Y movement and at the microscope (or with the software in STAGE menu - main toolbar).

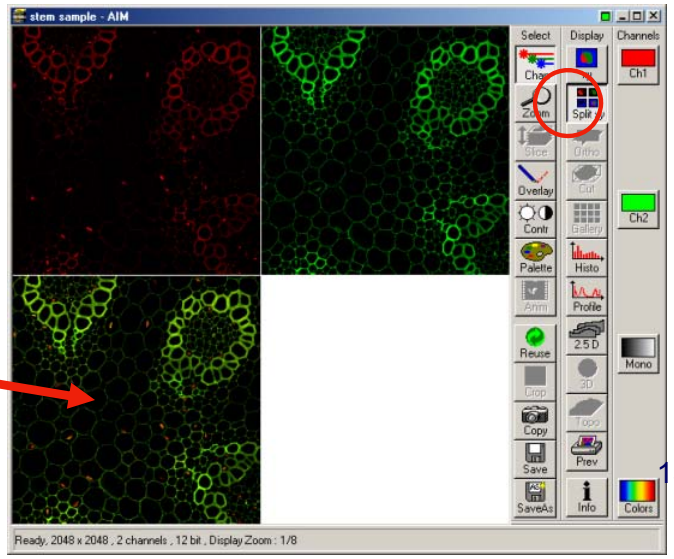
3) SINGLE records a single image.

4) STOP blanks the laser beam and stops the scanning mirrors.

5) Select CONT. for continuous scanning with selected scan speed.

6) On the image panel, XY shows a single large image with all channels. SPLIT XY displays a composite image of each channel and overlay.





G. Image Optimization

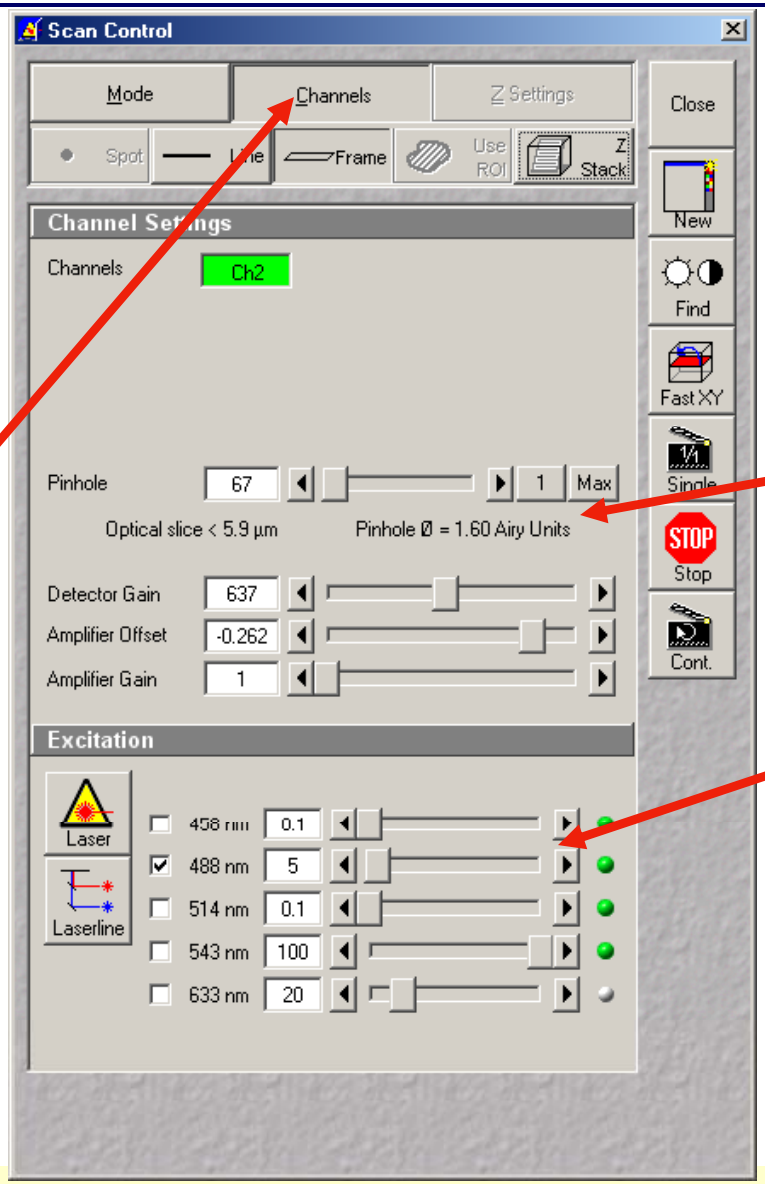
G-2. Adjust Channel Settings

1) Make sure the LSM and ACQUIRE buttons are selected on the main toolbar.

2) Select the SCAN button on the main toolbar.

3) Select CHANNELS. Use Channel Settings to adjust settings for each channel.

Additional wavelengths can be selected under LASERLINE.

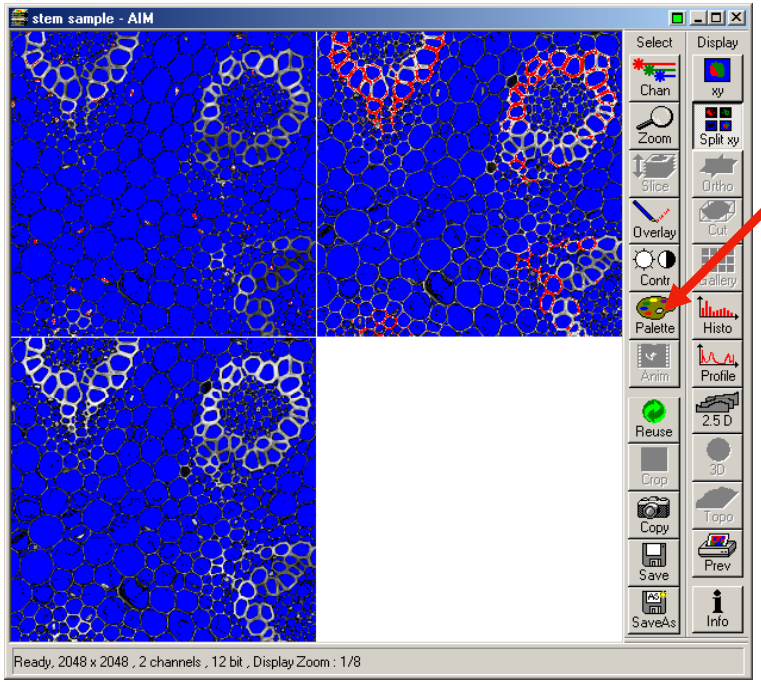


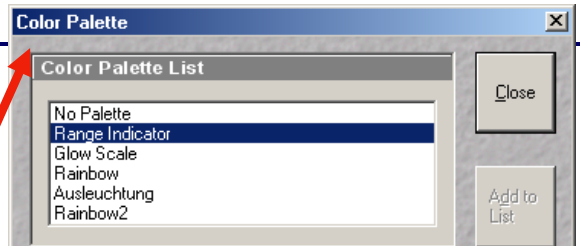
4) Use Pinholes to set pinhole size (typically, 1-1.5 Airy units).

5) Use Excitation to set transmission % for the laser lines you need.

NOTE: As a first approximation when setting excitation, the Ar lines should be set to ~5%. Setting 100% 543 and 20% 633 will result in 1 mW of excitation power.

G-3. Optimize Detector Settings



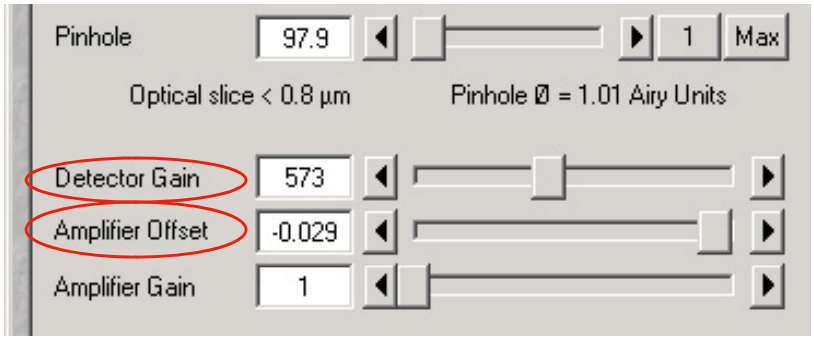


1) Select PALETTE.

2) This opens Color Palette List. Select *Range Indicator*. This table will show below-range pixels: in blue and saturation in red.

3) Select CONT. and adjust *Detector Gain* until few or no red pixels remain. Adjust *Amplifier Offset* until most blue pixels are just gone. Each channel must be adjusted independently (choose SPLIT XY to see each channel separately).

4) Select *No Palette* to return to normal channel display.



NOTE: If needed, you can use a higher *Amplifier Gain*. This will boost image intensity, but also significantly increase noise. You may also need to increase/decrease laser power.

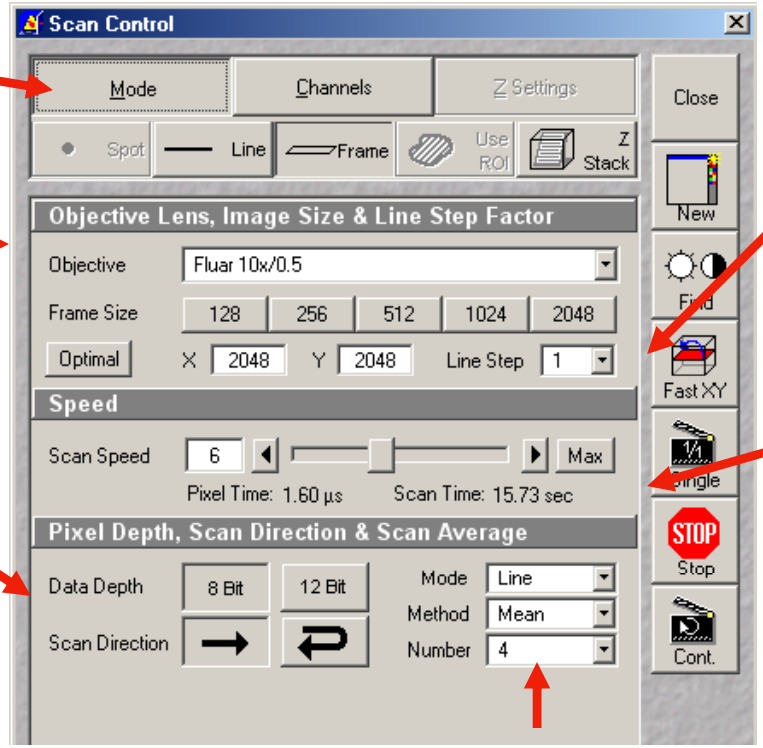
NOTE: In multi track, it's best done one channel at a time (only one laser line/one detector active). This will increase speed and reduce bleaching. Select/Deselect individual tracks in multi track mode on the CONFIG panel.

G-4. Optimize Scanning Parameters

1) Select MODE.

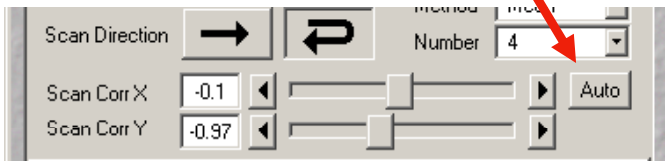
2) Objective Lens & Image Size. Select field size in pixels. OPTIMAL will give software recommendations (based on N.A. and λ).

5) Pixel Depth & Scan Direction. 8-bit of grey per channel &12 bit levels (publication quality images). Unidirectional scans require auto adjust.



3) Line Step. Scans every nth line.

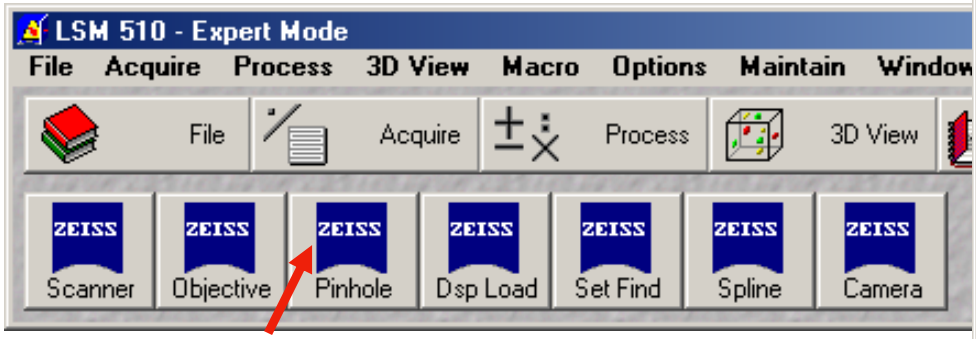
4) Scan Speed. Lower speed increases pixel dwell time: less noise, but more bleaching and longer imaging times.

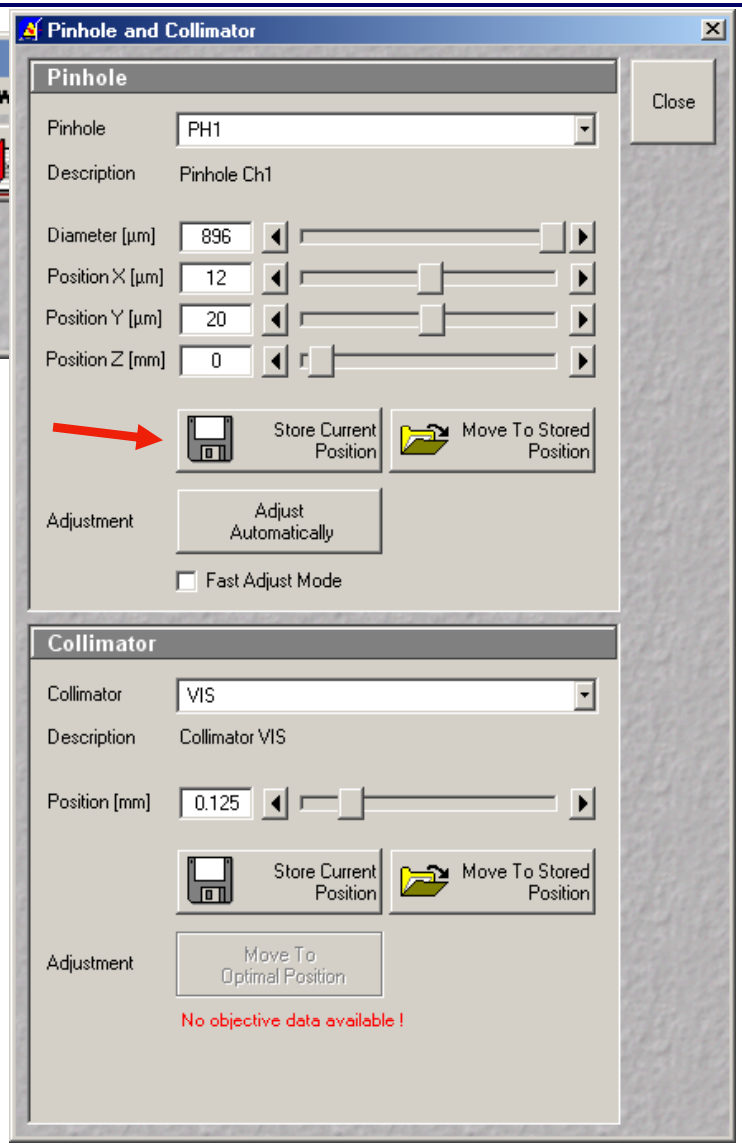


6) Scan Average. Line Average = each line scanned n-times, then pixels are averaged. Frame Average = complete frame scan n-times, then averaging. Mean = mean pixel value, Sum = pixel sum.

Hint: Weak samples - use sum instead of mean. Moving samples - use line averaging.

G-4. Optimize Pinhole (Advanced)





For greatest signal and resolution, realignment of the pinhole for each filter combination may be required.

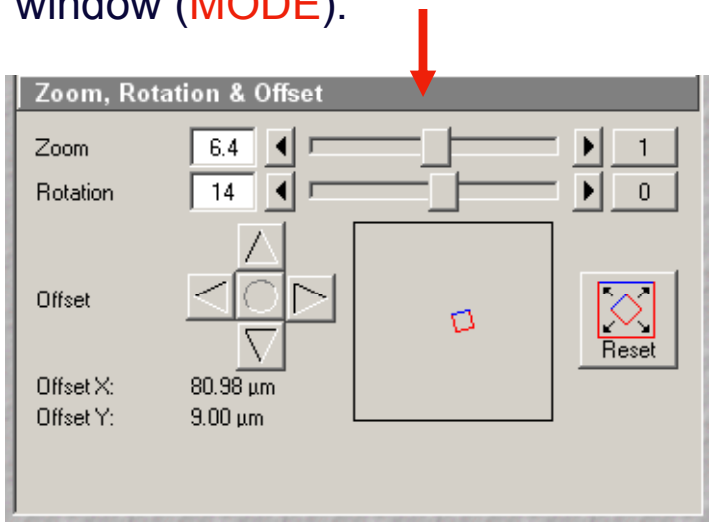
Gently nudge the X and Y positions to optimize signal while scanning.

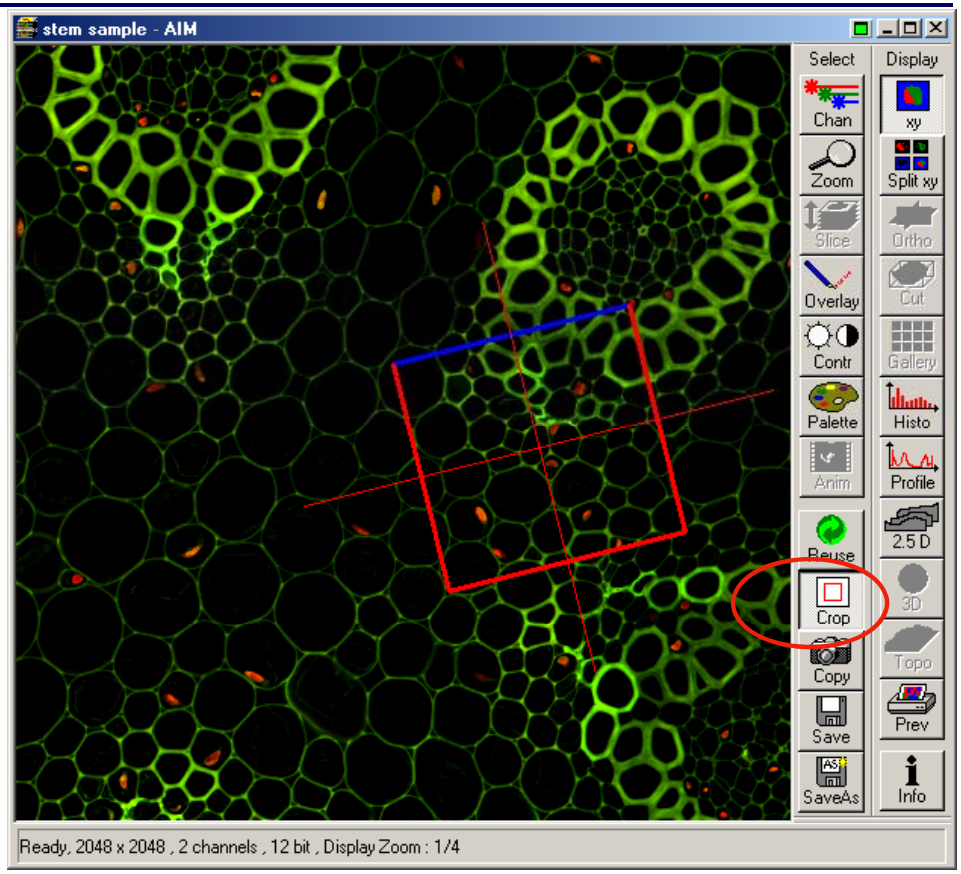
If successful, STORE POSITION

Alignment of the collimator is not advised

G-5. Adjust Optical Zoom

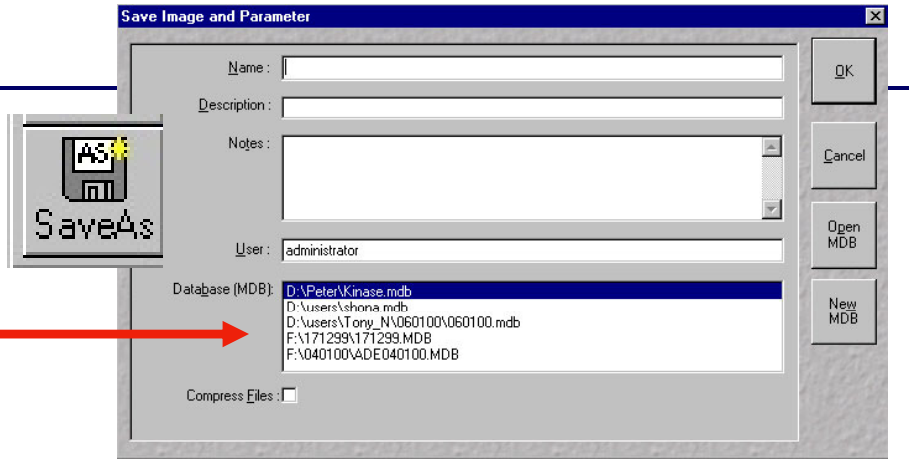
The scanner zoom allows you to "zoom in" on a region of interest and collect a magnified image. This is done with the CROP function in the image window, or by adjusting the Zoom, Rotation & Offset in the SCAN control window (MODE).





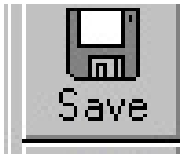
Zooming does affect image resolution (also sample bleaching). There is usually one optimal zoom setting for a given objective and image size, as described above. If you click OPTIMAL, the software will display the optimal image size for a given objective and zoom factor. Note that if you zoom, the optimal image size will decrease by the same factor.

H. Save Data

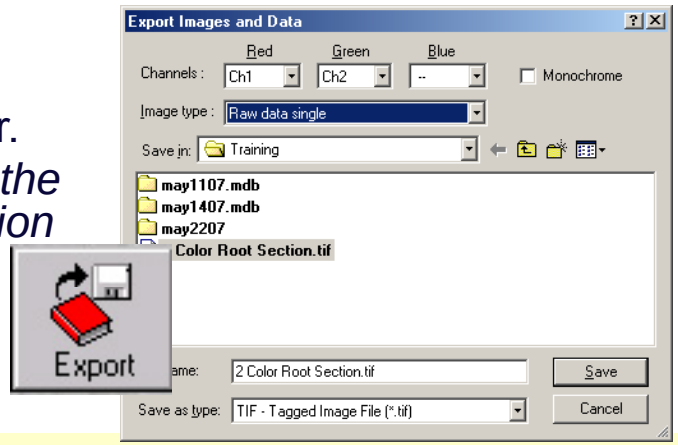


1) Saving Individual Images
   - Collect one image.
   - In the image window, click SAVE AS.
   - In the window Save Image and Parameters As, select a Database.

2) Auto Save Option
   - Go to OPTIONS>SETTINGS in main tool bar. Select the AUTOSAVE tab.
   - Make sure *Use LSM Image Database and Auto Increment Image Name* is selected.
   - Choose a Database.
   - Under *Base Image Name* enter desired file prefix (e.g. mmddyy).
   - Each file can now be saved using SAVE. The software will automatically increment the index digits.

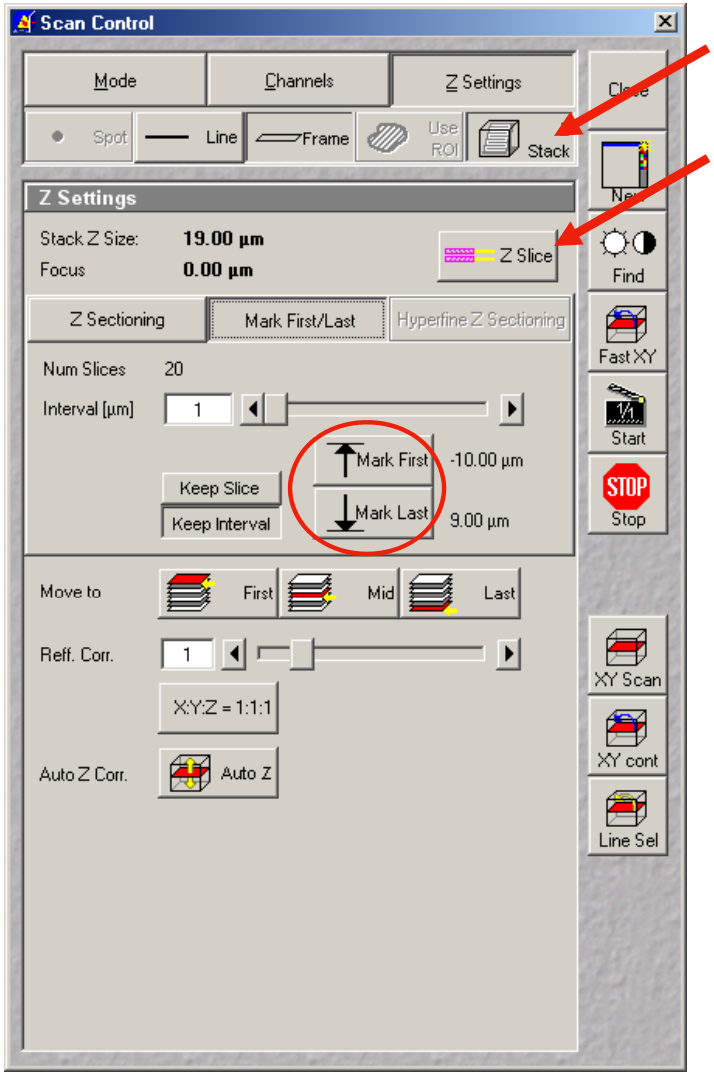


3) Exporting Data
   - Select FILE and then EXPORT on main tool bar.
   - Select *Raw data* (single or series), *Contents of the image window* (single or series) or *High resolution* (single or series).
   - Save as desired type (use 16-bit Tiff for 12-bit images).



I. Acquire a Z-series Stack

I-1. Mark First/Last Option

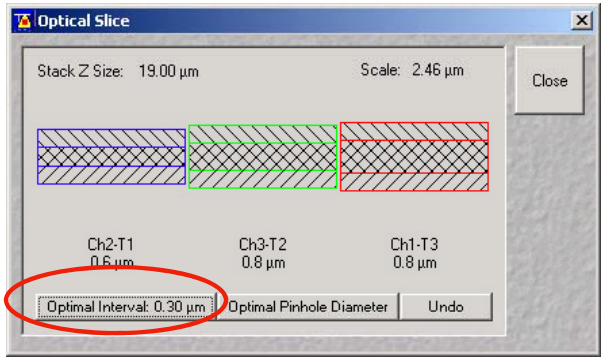


1) Select the Z STACK button under Z SETTINGS.

2) Select Z SLICE to open the Optical Slice control panel. OPTIMAL INTERVAL will set focus steps to the optimal value. OPTIMAL PINHOLE DIAMETER will give a fixed optical section for all λ's.

3) Select MARK FIRST/LAST.

4) Select XY CONT (or FAST XY) to start continuous scanning.



5) Use fine focus knob to find one edge of your sample – select MARK FIRST to define the beginning of stack.

6) Focus in the opposite direction until you find the other edge – then select MARK LAST.

7) STOP scanning and select START to collect Z-stack.

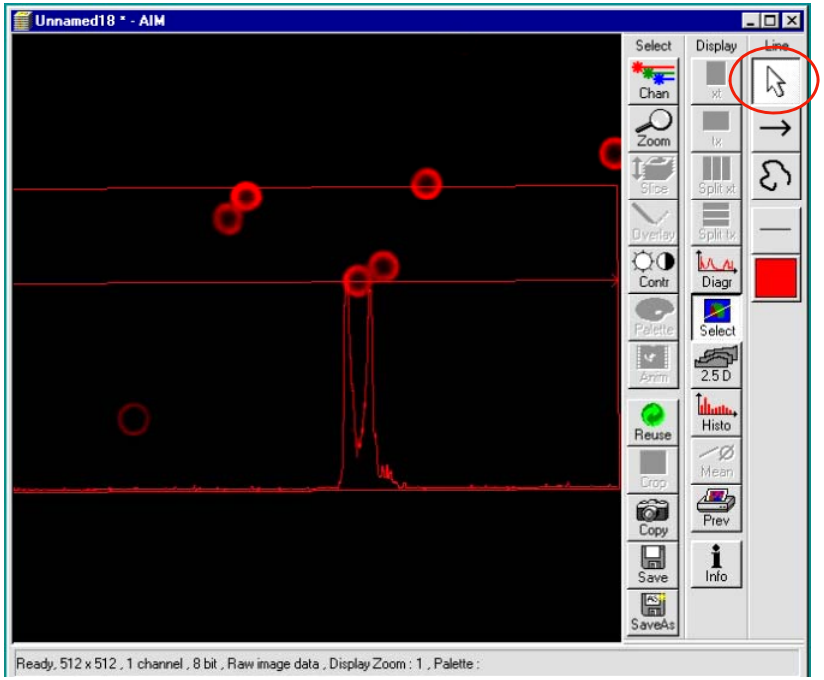
8) View entire Z-stacks using GALLERY on image panel, or individual sections using SLICE.

I-2. Z – Sectioning Option

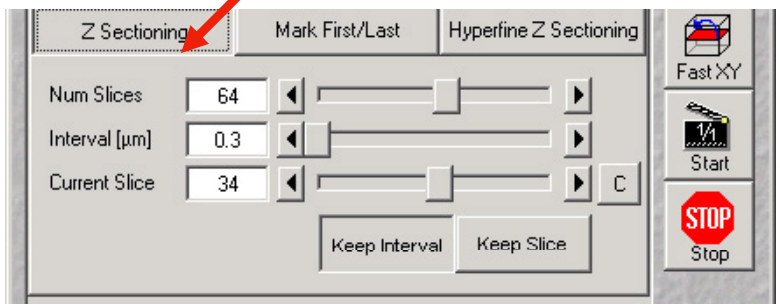
1) Select the Z STACK button under Z SETTINGS.

2) LINE SEL will collect an XY image.

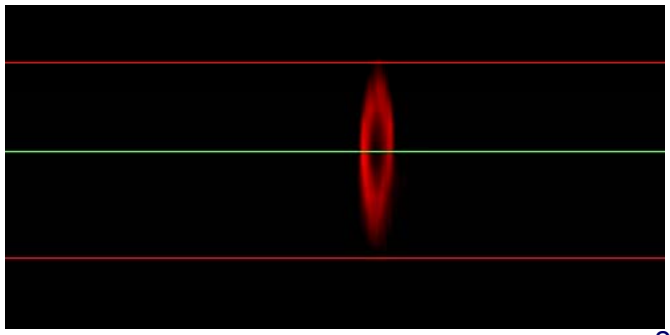
3) Use the arrow tool to define the XZ cut line (an area with good signal in the thickest part of the specimen).



4) Select Z SECTIONING button. Intervals and slices can be adjusted with KEEP INTERVAL (change # of slices) or KEEP SLICE (adjust interval).



5) RANGE will create XZ image of selected z range. Pull red lines to set limits for Z series – drag green line to change focus position.



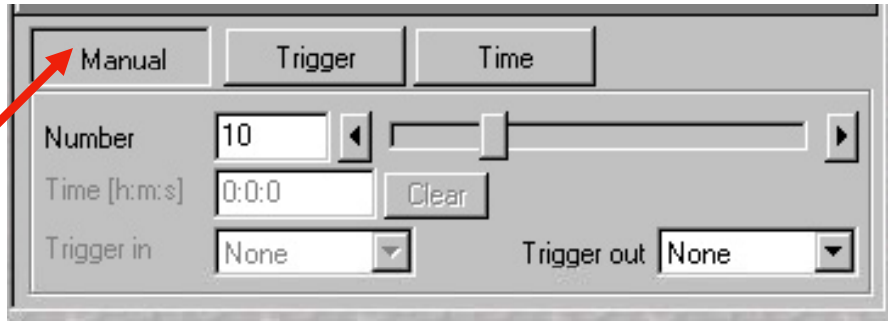
6) Select START to collect Z-stack.

J. Acquire a Time Series

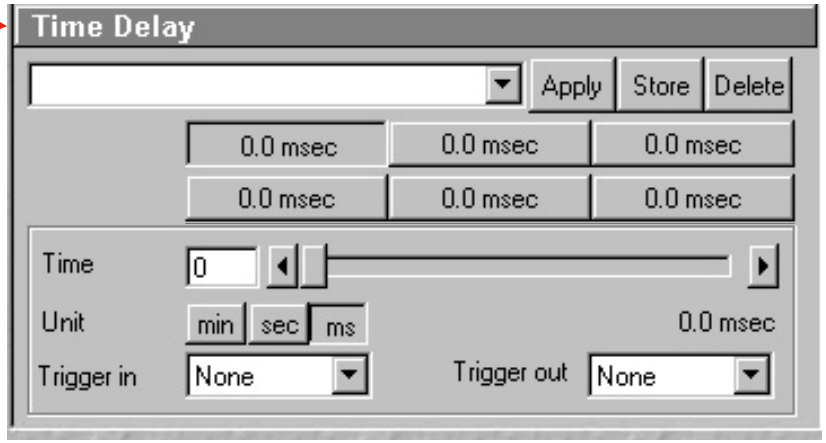
Before collecting a Time Series, set all parameters for collecting either a single XY section or a Z-stack. The Time Series macro will apply the current scanning settings.

1) Select TIME SERIES under ACQUIRE.

2) Under MANUAL enter the Number of cycles - total number of images you would like to collect. (Select TIME to start and stop at specific system times.)



3) Choose a delay between scans on the Time Delay panel.



4) Select START T to begin image acquisition.

5) View entire Time Series using GALLERY on image panel, or individual sections using SLICE.

k. Collect a DIC Image

K-1. Set up Kohler Illumination

1) Select VIS mode on main toolbar and then focus sample.

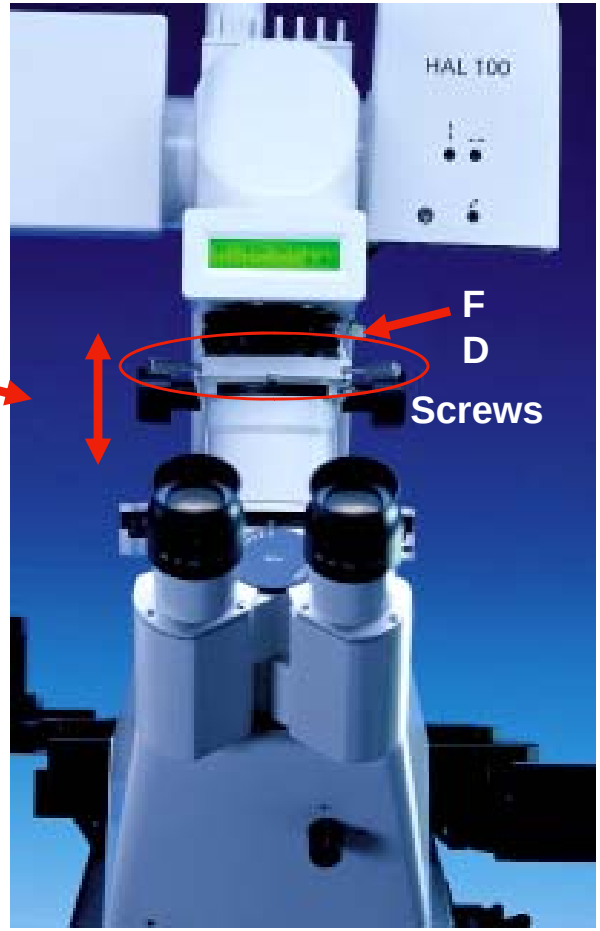
2) In BF, step down the field diaphragm to its minimum.

3) Raise or lower condenser to get diaphragm edge in focus.

4) Open diaphragm so it is at the edge of the field of view.

5) Center the diaphragm in the field of view using set screws on condenser tube.

6) Open diaphragm so it is just out of the field of view.



K-2. Image in VIS mode and PMT

1) Select condenser – DIC II for 20X, DIC III for 40X and 63X.

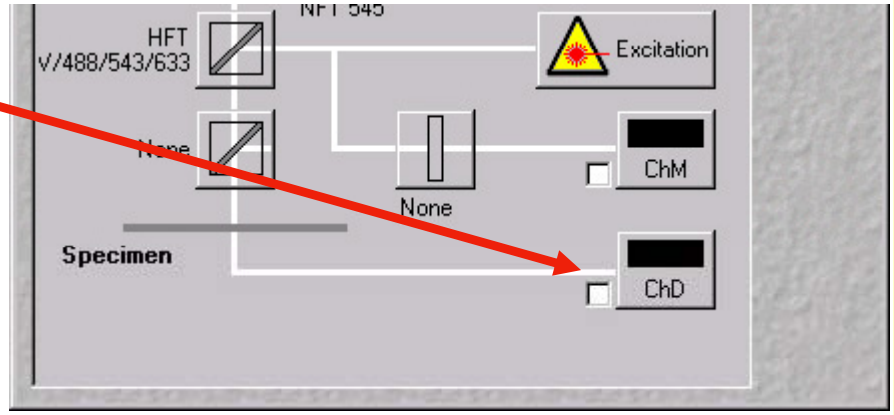
2) Make sure top analyzer is at 0º.

3) Maximize brightness with manual aperture (condenser tube).

4) Set reflector turret to DIC/BF. Make sure DIC, not BF, cube is in (check with NTUF staff), or use slide-in analyzer.

5) Rotate slider (on objective) to achieve best DIC image.

6) For PMT image select CHANNEL D in CONFIG panel.



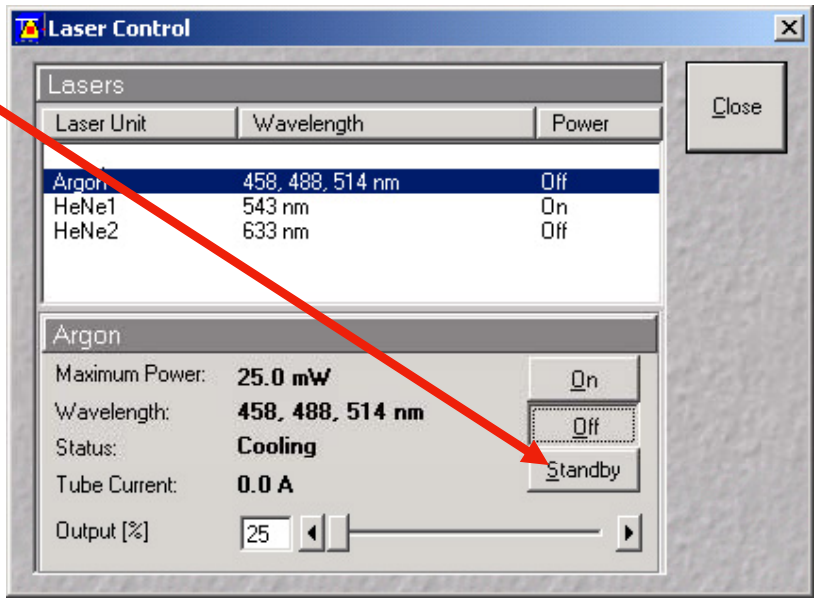
7) Use 543 nm laser line (for best results).

8) Manually adjust gain and amp offset (same as collecting a confocal image).

9) Adjust brightness with manual aperture.

L. Shut Down

1) Select ACQUIRE. Open the Laser Control panel and turn OFF the HeNe lasers. To turn off the Argon laser first click STANDBY, then reduce output power to 25%. Select OFF.



2) Wait for the Ar laser to go off by itself. The fan and purple light will go off and the status should read "connected" in the Laser Control panel.

3) Exit LSM 510 software and log off.

4) Wipe clean used immersion objectives with lens tissue. Clean up the microscope and monitor area.

5) Once the Ar laser fan has stopped switch OFF the remote control.

Warning: The remote control shuts down the entire system, including the laser power supply. Never turn OFF while Argon laser is still running!

6) Turn OFF the Hg vapor lamp and/or reflective HAL lamp and cover the microscope.

7) Sign blue log book and log usage on website.

Note: If someone is going to use the microscope after you, leave the HeNe lasers and the Hg lamp on, and set the Argon laser to "Standby." If you do leave the lasers on for the next person a dialog box will appear warning you that the system should not be shut down while lasers are ON - Click OK to get rid of box.